## CULTURE ADDITIVE, CULTURE MEDIUM AND CULTURE METHOD FOR ANIMAL CELLS

# CROSS REFERENCES TO RELATED APPLICATIONS

[0001] This application is a continuation of International Patent Application No. PCT/JP2019/038354, filed on Sep. 27, 2019, and claims priority to Japanese Patent Application No. 2018-184352, filed on Sep. 28, 2018, both of which are incorporated herein by reference in their entireties.

## BACKGROUND OF THE INVENTION

#### Field of the Invention

[0002] The present invention relates to additives for culturing, media for culturing, and methods for culturing animal cells that can improve proliferation ability of animal cells.

### Discussion of the Background

[0003] Many animal cells including stem cells such as embryonic stem cells, and induced pluripotent stem cells have been proliferated and maintained by adhesion culture using human-type recombinant matrix such as Matrigel, vitronectin and laminin as scaffold materials.

[0004] However, as a method for efficiently proliferating and culturing a large amount of animal cells for application to research, substance production, medical treatment, and the like, a method for suspension culturing in the state of a cell aggregate is widely used instead of the above-mentioned adhesion culture.

[0005] In culturing animal cells, it is generally necessary to add about 5 (w/v)% to 20 (w/v)% of serum to the culture broth to supplement components such as growth factor and the likes

[0006] However, serum is expensive, and contains not less than 500 kinds of proteins including unidentified components. Thus, it is often difficult to study at the molecular level the factors that act on cells and to isolate and purify substances produced from cells. In addition, serum may contain prions, viruses and the like. For culturing animal cells, therefore, a serum-free medium containing no serum or a low albumin medium with a decreased albumin content is often used. When culturing animal cells using a serum-free medium or a low albumin medium, proteins such as transferrin, various growth factors, hormones such as insulin, and the like are added to improve proliferation ability of the cells.

[0007] However, the kind and combination of growth factors, hormones, etc. necessary for proliferation vary depending on the type of animal cells to be cultured. It is thus difficult to obtain, at a low cost, an additive capable of promoting the proliferation of various animal cells.

[0008] Accordingly, a culture additive for animal cells that can efficiently promote proliferation of various animal cells has been demanded.

# SUMMARY OF THE INVENTION

[0009] Accordingly, it is one object of the present invention to provide culture additives, culture media and culture methods capable of improving proliferation ability of animal

cells particularly when culturing the animal cells using a serum-free medium or a low albumin medium.

[0010] These and other objects, which will become apparent during the following detailed description, have been achieved by the inventors' discovery that the proliferation ability of animal cells is improved by adding an amino acid such as tryptophan, serine, cysteine, cystine, methionine, arginine or the like, or glucose, or adding the aforementioned amino acid and glucose.

[0011] That is, the present invention provides the following:

- [0012] (1) An additive for culturing animal cells, comprising an amino acid or glucose, or an amino acid and glucose.
- [0013] (2) The additive of (1), wherein the amino acid is one or more kinds selected from the group consisting of tryptophan, serine, cysteine, cystine, methionine and arginine.
- [0014] (3) The additive of (1), wherein the amino acid is one or more kinds selected from the group consisting of tryptophan, serine, cysteine, cystine, methionine, arginine, histidine, isoleucine, leucine, lysine, phenylalanine and valine.
- [0015] (4) The additive of any of (1) to (3), wherein the animal cell is a stem cell.
- [0016] (5) The additive of (4), wherein the stem cell is one or more kinds selected from the group consisting of an adult stem cell, an embryonic stem cell, and an induced pluripotent stem cell.
- [0017] (6) The additive of any of (1) to (5), wherein the additive is for suspension culture of animal cells.
- [0018] (7) The additive of (6), wherein the suspension culture of animal cells is suspension culture in the state of cell aggregate.
- [0019] (8) The additive of any of (1) to (5), wherein the additive is for adhesion culture of animal cells.
- [0020] (9) A medium for culturing an animal cell, comprising an amino acid or glucose, or an amino acid and glucose, in addition to a medium component.
- [0021] (10) The medium of (9), wherein the amino acid is one or more kinds selected from the group consisting of tryptophan, serine, cysteine, cystine, methionine and arginine
- [0022] (11) The medium of (9), wherein the amino acid is one or more kinds selected from the group consisting of tryptophan, serine, cysteine, cystine, methionine, arginine, histidine, isoleucine, leucine, lysine, phenylalanine and valine.
- [0023] (12) The medium of any of (9) to (11), wherein the medium has a pH of not less than 7.
- [0024] (13) The medium of any of (9) to (12), wherein the animal cell is a stem cell.
- [0025] (14) The medium of (13), wherein the stem cell is one or more kinds selected from the group consisting of an adult stem cell, an embryonic stem cell and an induced pluripotent stem cell.
- [0026] (15) The medium of any of (9) to (14), wherein the medium is for suspension culture of animal cells.
- [0027] (16) The medium of (15), wherein the suspension culture of animal cells is suspension culture of animal cells in the state of cell aggregate.
- [0028] (17) The medium of any of (9) to (14), wherein the medium is for adhesion culture of animal cells.